Pure Appl. Biol., 12(1):769-778, March, 2023
http://dx.doi.org/10.19045/bspab.2023.120077

Research Article

Prevalence and antimicrobial susceptibility patterns of extended spectrum β-lactamase producers and non-producers *Pseudomonas aeruginosa* in Peshawar

Syed Jafar[1*], Naureen Ehsan Ilahi[2], Asad Ullah[3] and Fazal U Samad[1]
1. Department of Microbiology and Biotechnology, Abasyn University Peshawar, Khyber Pakhtunkhwa, Pakistan
2. Department of Biotechnology Virtual University of Pakistan, Rawalpindi, Pakistan
3. Center for Biotechnology and Microbiology, University of Peshawar, Peshawar, Pakistan
**Corresponding author's email: sjafarkhan303@gmail.com*

Citation
Syed Jafar, Naureen Ehsan Ilahi, Asad Ullah and Fazal U Samad. Prevalence and antimicrobial susceptibility patterns of extended spectrum β-lactamase producers and non-producers *Pseudomonas aeruginosa* in Peshawar. Pure and Applied Biology. Vol. 12, Issue 1, pp769-778. http://dx.doi.org/10.19045/bspab.2023.120077

Received: 10/11/2022 Revised: 11/01/2023 Accepted: 23/01/203 Online First: 10/02/2023

Abstract

Pseudomonas aeruginosa identified as one of the major pathogens responsible for nosocomial infections. The Extended Spectrum Beta Lactamases (ESBLs) enzymes production in *Pseudomonas* specie, have been rapidly reported all over the world. ESBLs usually known as the β-lactamase that is responsible for resistance against Penicillin and Cephalosporins. The current research was design for the Prevalence and Antimicrobial Susceptibility Patterns of Extended Spectrum β-Lactamase Producers and Non-producers *Pseudomonas aeruginosa* in Peshawar. Total of 1230 samples were taken, in which 105 (8.53%) showed positive result for *P. aeruginosa* growth. Samples taken from females showedhigher rate (61.9%) of *P. aeruginosa* occurrence as compared to male (48.3%). *P. aeruginosa* 46.6%, were isolated from pus, 31.4% urine, 11.4% wounds, 8.5% swabs (ear, throat) and 1.9% from High Vaginal Swabs (HVS). Most of the isolates were sensitive to Carbapenems (Meropenem 84%) followed by Aminoglycosides (Amikacin 73.3%), Beta- Lactamse Inhibitor (Cefoperazone+Sulbactum 69.5%), Cephalosporins (Cefepime 60.9%), Fosfomycin (Fosfomycin 57.1%) and Fluoroquinolones (Ciprofloxacin 56.1%). Out of 105 *P. aeruginosa* isolates, 46 (43.8%) were ESBLs producers and 49 (46.6%) were non-ESBLs producers. It concluded that Meropenem (MEM) 82.6% and Imipenem (IMP) 80.43% showed greater sensitivity against both ESBLs-positive and non-ESBLs-negative *P. aeruginosa* among selected antibiotics. While, the Amoxicillin-Clavulanic acid and Cefotaxime were the least efficient drugs with a higher number of samples (88.5% and 80.9% respectively) showed resistance. The ESBLs-producing isolates showed more resistance as compared to the Non-producing *P. aeruginosa* isolates. Meropenem and Imipenem were found the most effective antibiotics.

Keywords: Antibiotics; Drug resistance; ESBLs; HAIs; Opportunistic pathogen; *Pseudomonas aeruginosa;* Sensitivity; Susceptibility.

Introduction

P. aeruginosa is gram negative, motile, aerobic rod-shape bacteria which is one of the major pathogens responsible for nosocomial infections. *P. aeruginosa* is normally present in human normal flora in healthy individual; usually colonize on skin surface and gastrointestinal tract. *P. aeruginosa* is the causative opportunistic pathogen in immune compromised patients having cystic fibrosis,

cancer and thermal burns. It presents in particularly in chronic patients and phenotypically very unstable [1, 2]. *Pseudomonas* has been rapidly reported pathogen responsible for a variety of severe Hospital Acquired Infections (HAIs), mostly in patients having weak immune responses. Colonization occurrence is repeatedly common in health care centers, especially on medical equipment, beds and water Colonization occurrence are repeatedly common in hospitals, especially on medical equipment, disinfectants bedding and water [1]. *P. aeruginosa* is responsible for causing Urinary Tract Infection (UTIs), dermatitis, otitis, conjunctivitis, Gastro Intestinal Tract (GIT) infection and infections related to joints and bones [3]. Humans and millions of other species on earth facing climate crisis due to rapid climate change. Climate change effect the human health by different ways like heat mortality, food effects, crops, rising water level, transmission of microbial infections and floods [4]. Globally the most important and alarming issues regarding to human health is the increase and spreads of antibiotic resistant microorganisms. Antibiotics are one of the main pillars against infections in present health care systems [5]. The climate change is directly linked with antibiotics resistance, because of major climate change the microorganisms also modified their self-according to change in their environment [6]. Human activities play an important role in the emergence and spread of resistant pathogens in ecosystems includes misuse and discharge of antibiotics, discharge of resistant pathogens in healthcare waste, research laboratories and pharmaceutical industries waste [7]. *P. aeruginosa* is one of the most common resistant bacteria against β- lactams antibiotics. It is an opportunistic pathogen responsible for verity of hospital acquired infections [5]. Among the resistance bacteria, *P. aeruginosa* resistance strains are rapidly reported around the globe [4]. The most alarming threat related to *P. aeruginosa* is the multidrug resistance and only limited numbers of antimicrobial agents are effective against the pathogen. The Extended Spectrum Beta Lactamases (ESBLs) enzymes production in *Pseudomonas* specie, have been rapidly reported all over the world. These enzymes have the capability to hydrolyze and inactivate a wide range of antibiotics. ESBLs are the enzymes responsible for resistance against various Carbapenem, Penicillin and Cephalosporin [3]. *P. aeruginosa* gain drug resistance against the beta-lactam antibiotics by several mechanisms like β-lactamases production, reducing permeability of membrane, Penicillin Binding Proteins (PBP) affinity and modification of enzymes by plasmid-mediated resistance [8, 9]. The ESBLs-producers are more resistant as compared to the non-producers due to the correlation between virulence factors and production of ESBLs enzymes against antimicrobial agents [10]. Gaining additional genes by horizontal gene transfer is another mechanism for antibiotic resistance acquisition [11]. In case of antibiotic resistance, major cause of resistance is over dependency on antibiotics and over use of antimicrobials [12]. The aim of this study is to determine the Prevalence and Antimicrobial Susceptibility Patterns of ESBLs positive and negative *P. aeruginosa* strains in different clinical samples collected from Khyber teaching hospital (KTH) and lady reading hospital (LRH) Peshawar. It also focused on the comparative study of susceptibility patterns of ESBL's producing and non-ESBL's producing *P. aeruginosa* to determine their resistance capability and provide data to formulate an effective drug therapy.

Materials and Methods

Sample collection

Samples of urine, pus, bodily fluids, wounds and swabs were collected from KTH and

LRH Peshawar by using sterile bottles, cotton swabs, and syringes [13]. Samples were taken from both Out-Patient Department (OPD) and In-Patient Department (IPD). These were transferred from hospitals to Microbiology Research Laboratory of Abasyn University by following standard protocols.

***Pseudomonas aeruginosa* Isolation**

Bacterial culture was prepared by using MacConkey, Blood and Cystine-Lactose-Electrolyte-Deficient (CLED) agar. The MacConkey agar was used as a selective media for Gram-negative bacteria. *P. aeruginosa* identified as non-lactose fermenter on MacConkey agar. CLED agar was used as differential media for urinary tract microbes. Samples were streaked on the media and the inoculated media were incubated for 24 hours at 37°C. After that bacterial colonies were gone through gram staining to differentiate into gram positive and negative. Different biochemical tests like triple sugar iron, catalase, coagulase, citrate, urease, oxidase and indole were performed for further classification and bacterial identification.

ESBLs detection

Screening test

Screening test was done for detection of ESBLs production by using Cefpodoxime, Ceftazidime, Aztreonam, Cefotaxime and Ceftriaxone antibiotic discs as per CLSI guidelines. Nutrient broth was used to prepared inoculum and incubated for 24 at 37°C. The turbidity was adjusted to 0.5 MacFarland standards. Thereafter, it was uniformly swabbed on to Muller-Hinton Agar (MHA) plates using a sterile cotton swab. Using sterile forceps, antibiotics discs were applied on MHA plate surface and incubated at 35-37°C for 18–24 hours. Zones of inhibition were observed for interpretation. Resistance to at least one of the mentioned antibiotics was interpreted as positive.

Double disc synergy test

Double Disc synergy method was used for detection of ESBLs production in positively screened samples. The antibiotic discs of Ceftriaxone (CRO 30 µg), Cefotaxime (CTX 30 µg), Ceftazidime (CAZ 30 µg) and Aztreonam (ATM 30 µg) were placed at 20mm from Amoxicillin+Clavulanic acid (AMC=20+10µg). Clavulanic acid + Amoxicillin were put in the center of MHA plates following the CLSI recommendations. After18-20hrs' incubation, cleared zones of third-generation Cephalosporins extended towards disc of Amoxicillin + Clavulanic acid, were considered as ESBLs produces.

Phenotypic confirmatory disc diffusion test

Phenotypic confirmatory test used for the confirmation of ESBLs producers. A combination disc Ceftazidime + Clavulanic acid (30 µg +10 µg) was placed on MHA, then Ceftazidime (30µg) was placed on the surface of MHA plate at a distance of 15-20mm. By following overnight incubation at 37^{o}C, if the inhibition zone of Ceftazidime + Clavulanic acid was ≥5mm as compare to Ceftazidime alone, then the isolate considered as ESBLs producer.

Antibiotics sensitivity testing of ESBLs and non-ESBLs

Antibiotic sensitivity tests were carried out using Kirby-Bauer disk diffusion method [14]. MHA was used for sensitivity analysis. About 3 to 5 colonies of test organisms were dispersed in 5 ml of nutrient broth and mixed slowly. The media broth incubated at 35 to 37°C for overnight. The turbidity of the media broth adjusted to 0.5 McFarland standards. Then the standardized Inoculum was swabbed uniformly on the MHA plates. The antimicrobial discs were applied using sterile pincers on the MHA surface, media plates were incubated for 24hours at 35 to 37°C and zone of inhibitions were measured carefully. A total 13 antibiotics were applied in the sensitivity testing**.**

Results

Present study was conducted in Microbiology

Research Laboratory, Abasyn University Peshawar. Total of 1230 clinical samples taken from Lady Reading Hospital (LRH) and Khyber Teaching Hospital (KTH) Peshawar.

Out of 1230 samples, 105 (8.53%) were positive for *P. aeruginosa* growth. In 105 *P. aeruginosa* isolates, 46 (43.8%) were detected as ESBLs producers and 49 were non-ESBLs producers. Samples taken from females showed 27/46 (58.69%), while male were 19/46 (41.30%). In 49 *P. aeruginosa* positive pus samples, 22/49 (44.89%) were ESBLs producer, 14/33 (42.42%) in urine, 7/12 (58.33%) wounds, 1/9 (11.11%) swabs (ear, throat), 2/2 (100%) and High Vaginal Swabs (HVS) as shown in (Table 1).

Table 1: Distribution of ESBLs-producers and non-producers *P. aeruginosa* samples in various specimens

Clinical samples	Total	ESBLs-producers N (%)	Non- ESBLs producers N (%)
Pus	49	22 (44.89%)	27 (55.10%)
Urine	33	14 (42.42%)	19 (57.57%)
Wounds	12	07 (58.33%)	05 (41.66%)
Swabs (ear, throat)	09	01 (11.11%)	08 (88.88%)
High Vaginal Swabs (HVS)	02	02 (100%)	00 (00)
Total	105	46	49

Results observed the frequency of ESBLs in different age groups patients having *P. aeruginosa* infections. Patents having age of 31years or above showed higher occurrence 21/46 (45.6%) followed by 21-30 years 12/26 (46.1%), 11-20 years 11/26 (42.3%) and 0-10 years 2/7 (28.5%). Gender base results showed higher numbers of ESBLs occurrence in females 27(58.6%) as compared to males 19(41.3%) (Fig. 1).

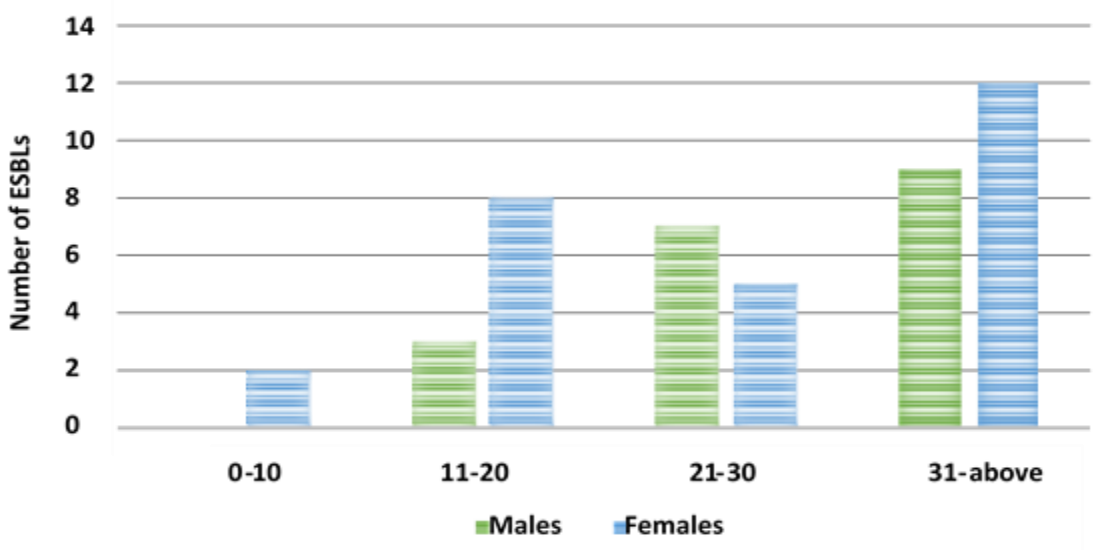

Figure 1: Distribution of ESBLs-producers P. aeruginosa on gender basis in various age group

Comparative results showed that, the susceptibility rate of most antibiotics against non- ESBLs producers were high as compare with the ESBLs producers (Fig. 2). Carbapenems (IMP, MEM) showed best susceptibility against both the ESBLs producers and non-ESBLs producers. Meropenem (MEM) was 82.6% susceptible against ESBLs producers and 86.4% against the non-ESBLs. Amikacin (AK) from class Aminoglycosides were 69.5% susceptible against ESBLs producers and 76.2% to non-ESBLs producers isolates. Cefoperazone+ Sulbactum (SCF) are the Beta-Lactamase inhibitors which showed good susceptibility against both the the ESBLs 69.5% and non-ESBLs 67.7%. Aztreonam (ATM) from class Monobactam was observed 58.6% to ESBLs producers and 83% to non-ESBLs producers. Fluroquinolones are widely used against gram negative bacteria; results showed Ciprofloxacin (CIP) were 36.9% susceptible to ESBLs and 71.1% to non-ESBLs respectively. Cephalosporins are one of the antibiotics class highly used against *Pseudomonas* species. Cephalosporins (CAZ, CTX, CRO, and FEP) were observed with susceptibility 19.5%, 23.9%, 10.8% and 58.6% against ESBLs and 35.5%, 18.6%, 49.1%, and 62.7% to non-ESBLs *P. aeruginosa* isolates (Fig. 3).

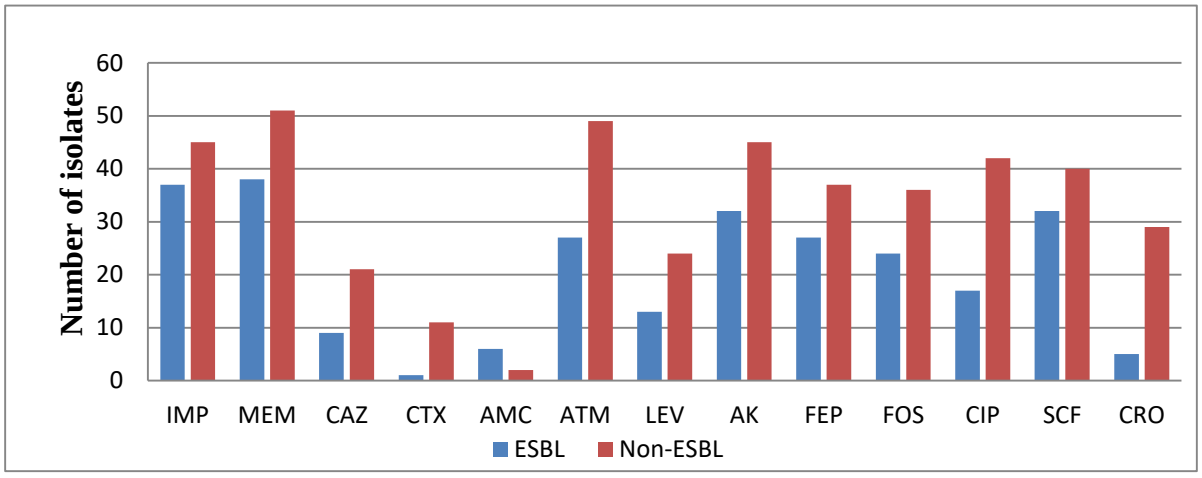

Figure 2: Comparative sensitivity of ESBLs-producers and non-ESBLs producer's isolates against selected antibiotics

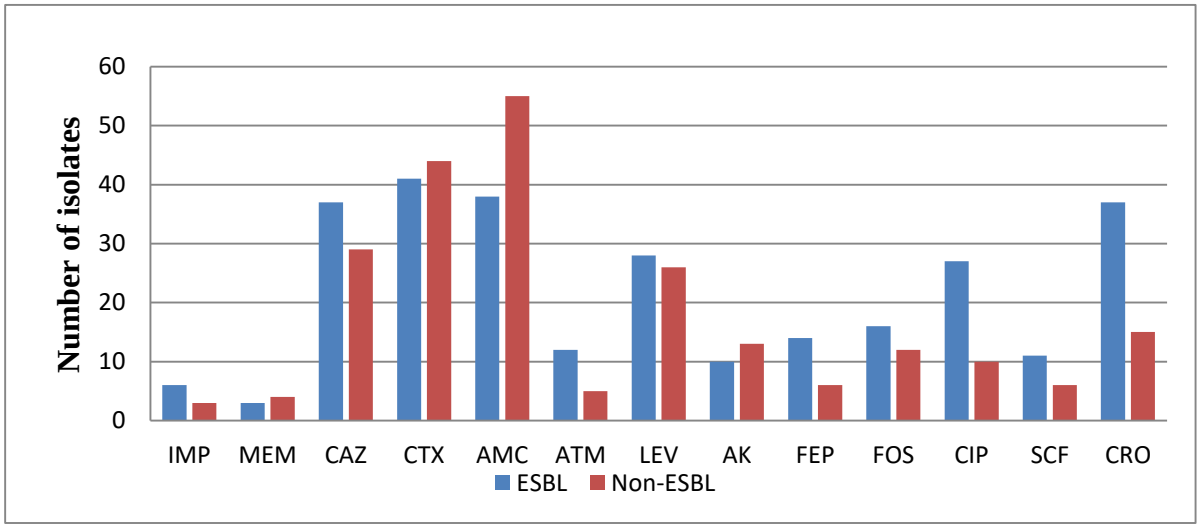

Figure 3: Comparative resistance of ESBLs-producers and non-ESBLs producers isolates against selected antibiotics

The ESBLs-producers showed more resistance against all the selected antibiotics, contrary to non- ESBLs producers (Fig. 3.3). The 3rd Generation Cephalosporins (CAZ, CTX and CRO) were highly resistant 80.4%, 89.1% and 80.4% against the ESBLs producers and 49.1%, 74.5% and 25.4% in non-ESBLs producers. Between the beta-lactamase inhibitors, Amoxicillin+Clavulanic acid (AMC) observed highly resistant to ESBLs 82.6% and 93.2 to the non-ESBLs isolates. Levofloxacin (LEV) from class Fluroquinolones were 60.8% resistant against ESBLs producers and 44% to the non-ESBLs producers. Carbapenems (MEM, IPM) were the class of antibiotics found highly active and having the lowest resistance rate against both ESBLs 6.5%, 13% and non-ESBLs 8.4%, 5% respectively **(**Table 2**)**. Results found that the ESBLs producing isolates were more resistant as compared to the non-ESBLs producing isolates against the selected antibiotics.

Table 2: Comparative analysis susceptibility patterns of ESBLs-positive and Non-ESBLs negative *P. aeruginosa* against selected antibiotics

	ESBLs			Non-ESBLs		
Antibiotics	**Sensitive N(%)**	**Intermediate N(%)**	**Resistant N(%)**	**Sensitive N(%)**	**Intermediate N(%)**	**Resistant N(%)**
IMP	37(80.43)	03(06.52)	06(13.04)	45(76.24)	11(18.64)	03(05.08)
MEM	38(82.60)	05(10.86)	03(06.52)	51(86.44)	04(08.47)	04(08.47)
CAZ	09(19.56)	00(00)	37(80.43)	21(35.59)	09(15.25)	29(49.15)
CTX	01(23.91)	04(02.17)	41(73.91)	11(18.64)	04(06.77)	44(74.57)
AMC	06(13.04)	02(04.34)	38(82.60)	02(03.38)	02(03.38)	55(93.22)
ATM	27(58.69)	07(15.21)	12(26.08)	49(83.05)	05(08.47)	05(08.47)
LEV	13(28.26)	05(10.86)	28(60.86)	24(40.67)	09(15.25)	26(44.06)
AK	32(69.56)	04(08.59)	10(21.73)	45(76.27)	01(01.69)	13(22.03)
FEP	27(58.69)	05(02.17)	14(39.13)	37(62.71)	16(27.11)	06(10.16)
FOS	24(52.17)	06(13.04)	16(34.78)	36(61.01)	11(18.64)	12(20.33)
CIP	17(36.95)	02(04.34)	27(58.69)	42(71.18)	07(11.86)	10(16.94)
SCF	32(69.56)	03(06.52)	11(23.91)	40(67.79)	13(22.03)	06(10.16)
CRO	05(10.86)	04(08.69)	37(80.43)	29(49.15)	15(25.42)	15(25.42)

Discussion

Pseudomonas aeruginosa is one of major pathogen responsible for nosocomial infections. Current study is focused on the prevalence of ESBLs producers and non-producers *P. aeruginosa* and compare its antimicrobial resistance profile to provide an insight into formulating effective antibiotic strategy.

Antibiotic resistance is major problem in antibiotic therapies and is a serious concern to the human health globally. The β-lactam is one of the major group antibiotics which is commonly used against the bacterial infections. The β-lactam antibiotics contain a β-lactam ring in their molecular structure which inhibits the synthesis of bacterial cell wall. Several bacterial species have the ability to resist against these antibiotics by adopting different strategies. The resistance procedure against the beta-lactam antibiotics includes, beta-lactamase production, reducing membrane permeability, the altered affinity of target Penicillin binding proteins and plasmid mediated resistance involving modifying enzymes. Production of extracellular enzymes β- lactamases is the emerging health problem world widely. Bacterial species is capable to produce different types of β-lactamases like, Amp C beta-lactamase, Metallo Beta-Lactamase (MBLs) and Extended Spectrum Beta Lactamase (ESBLs) against antibiotics. Among these enzymes, the most important

resistance emerging enzymes is ESBLs in *Pseudomonas* species. ESBLs break down the β-lactam ring in various β-lactam drugs and make the drugs ineffective to change in the molecular structure. In present study, out of 105 isolates 46(43.8%) were reported as ESBLs- producers. The positivity rate was higher in females 58.69%, as compare to males 41.30%. The ESBLs were found in different age groups, Patents having age of 31years or above showed higher occurrence (45.6%) followed by 21-30 years (46.1%), 11-20 years (42.3%) and 0-10 years (28.5%) results are comparably similar to other relative studies **[15, 16]**. The outcomes of these results showed 35.8%,44.3%, 30%,22.2%, 25% and 45.6% isolates as ESBLs- producers and previous studies like **[17-20]**. Moreover, antibiotics resistance in ESBLs producer strains is greater than the non-ESBLs producer strains of *P. aeruginosa* which supports the study conducted by **[18]**, at Menofia, Egypt and Peshawar, Pakistan **[16]**.

The present work shows that ESBLs-producers are more resistant as compared to the non-producers which are supported by **[10]** study that, due to the correlation between virulence factors and production of ESBLs enzymes against antimicrobial agents. The resistance procedure against the beta-lactam antibiotics includes, beta-lactamase production, reducing membrane permeability, the altered affinity of target Penicillin binding proteins and plasmid mediated resistance involving modifying enzymes **[8, 9]**. Amoxicillin-Clavulanic acid is a combination antibiotic used for a number of bacterial infections. It consists of a β-lactam antibiotic, Amoxicillin and potassium Clavunate, the β- lactamases inhibitors. Due to excessive and uncontrolled use of Amoxicillin-Clavulanic, the drug facing high resistance which increasing rapidly, which showed in current study the *P. aeruginosa* isolates showed higher resistance 88.5% against Amoxicillin-Clavulanic acid, and also in previous studies like 87% at Menofia, Egypt **[18]**, 98.04% at Kohat, Pakistan **[21]** and 83.8% at Multan, Pakistan **[22]**. Cephalosporins are the β-lactam antibiotics; their molecular structure is basedon β-lactam ring. B-lactamases enzymes hydrolyzed the amide bonds of the ring and make the Cephalosporins ineffective **[3]**. ESBLs are one of important B-lactamases enzyme, which shows high resistance against the Cephalosporins as showed in present study. In current study 80.9% *P. aeruginosa* isolates showed resistance against Cefotaxime, 62.8% Ceftazidime and 49.5% to Ceftriaxone. Present study results related with other studies in which 91.4% strains were resistant to Ceftazidime, 78.7% Cefotaxime and 82.9% to Ceftriaxone at Saudi Arabia **[20]**, 86.6% Ceftriaxone and 76.6% Cefotaxime at Karachi, Pakistan **[17]**. Aminoglycosides are the group of antibiotics mainly used against gram-negative rods bacterial infections. But in recent studies various microbes' shows resistance to these antimicrobial drugs. Amikacin is one of these antibiotics which is very effective in past, but gradually many bacterial species showed resistance to the drug. In current study, the results showed 21.9% isolates were resistance to Amikacin, which is related to previously work done on similar subject like 10% at Karachi, Pakistan. The comparison of study done by at Karachi and present study showed an increased resistance to Amikacin, due to misuse of these antibiotics **[17]**.

Ciprofloxacin belongs to Fluoroquinolones, which are commonly used for gram-negative bacterial infections. In current study results 56.1% isolates were sensitive to Ciprofloxacin and having comparable results with the previous studies like 73.6% at Tehran, Iran **[23]** and 49% at Peshawar, Pakistan **[16]**. Cefoperazone-Sulbactam is combination drug mostly used for Urinary Tract Infections (UTI). The Cefoperazone-

Sulbactam contains Cefoperazone as β-lactam antibiotic and Sulbactam, which is the β-lactamases inhibitor drug. Present study showed 69.5% of *P. aeruginosa* isolates were sensitive to Cefoperazone-Sulbactam. These results showed similarity to previous work 74% reported by **[24]** at Varanasi, India and 69.1% by **[15]**, at Peshawar, Pakistan, which indicates the Cefoperazone- Sulbactam is still effective. Carbapenems are class of drugs commonly used for the treatment for high risk infections, in current study the sensitivity of Meropenem and Imipenem were reported 84.7% and 78.09% respectively. These results showed lower sensitivity compared to studies of **[16]** reported 99% Meropenem and 96% Imipenem, **[15]** showed 91.02% Meropenem and 84.4% Imipenem, **[25]** reported 91.5% Meropenem and 78.5% Imipenem. Comparison of these studies and present study shows that the sensitivity of Carbapenems decreases and there is gradual increase in resistance, which is an alarming situation to public health.

Current study showed that a number of β-lactam drugs slowly became ineffective to *P. aeruginosa*, especially against ESBLs producers. Due to increase and rapid spread of MDR strains of *P. aeruginosa* in health care centers makes an alarming situation. Carbapenems were the most effective drugs with high percentage of sensitivity and lower resistance percentage. Meropenem showed high sensitivity of 84.7% and the Imipenem were 78.09%. The Meropenem and Imipenem are the drugs of choice against *P.aeruginosa*.

Conclusion

Compared to earlier studies, we observe that the effectiveness of several antibiotics rapidly declines over time. According to Present study ESBLs-producing isolates isolate were 43% in *P. aeruginosa*, which are highly increased as compared to previous studies. High resistance showed by ESBLs-producers as compare with non-ESBLs producers and therefore clear difference can be seen in their susceptibility profile. Meropenem and Imipenem were found the most effective antibiotics.

Authors' contributions

Designed and Conceived the experiments: S Jafar & N llahi, Experiments Performed: S Jafar & A Ullah, Data Analysis: FU Samad & A Ullah, contributed in materials, reagents, tools: A A Ullah, FU Samad & S Jafar, Analyzed the data: FU Samad & K Bashir, Wrote the paper: S Jafar.

References

1. Karami P, Mohajeri P, Mashouf RY, Karami M, Yaghoobi MH, Dastan D & Alikhani MY (2019). Molecular characterization of clinical and environmental Pseudomonas aeruginosa isolated in a burn center. *Saudi J Biol Sci* 26(7): 1731-1736.
2. Martina CA, Ifeoma ME, Maurice NO, Beatrice OO, Chinelo CE & Chizoba, AO (2019). Phenotypic and molecular characterization of extended spectrum Î²-lactamase producing Pseudomonas aeruginosa in Nigeria. *Afr J Biotechnol* 18(32): 1083-1090.
3. Aggarwal R, Chaudhary U & Bala K (2008). Detection of extended-spectrum β-lactamase in *Pseudomonas aeruginosa*. *Indian J Pathol Micr* 51(2): 222.
4. Burnham JP (2021). Climate change and antibiotic resistance: a deadly combination. *Ther Adv in Infec Dis* 8: 2049936121991374.
5. Zarei-Baygi A & Smith AL (2021). Intracellular versus extracellular antibiotic resistance genes in the environment: Prevalence, horizontal transfer, and mitigation strategies. *Bioresource Technol* 319: 124181.
6. Molnar A (2019). Antimicrobial resistance awareness and games. *Trends Microbiol* 27(1): 1-3.
7. Subramanya SH, Bairy I, Metok Y, Baral BP, Gautam D & Nayak N (2021).

Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. *Sci Rep-UK* 11(1): 1-13

8. Aendekerk S, Diggle SP, Song Z, Høiby N, Cornelis P, Williams P & Camara M (2005). The MexGHI-OpmD multidrug efflux pump controls growth, antibiotic susceptibility and virulence in *Pseudomonas aeruginosa* via 4-quinolone-dependent cell-to-cell communication. *Microbiol* 151(4): 1113-1125.
9. Tomás M, Doumith M, Warner M, Turton JF, Beceiro A, Bou G & Woodford N (2010). Efflux pumps, OprD porin, AmpC β-lactamase, and multiresistance in Pseudomonas aeruginosa isolates from cystic fibrosis patients. *Antimicrob Agents Ch* 54(5): 2219-2224.
10. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, Van Duin D & Clancy CJ (2021). Infectious Diseases Society of America guidance on the treatment of extended-spectrum β-lactamase producing Enterobacterales (ESBL-E), carbapenem-resistant Enterobacterales (CRE), and Pseudomonas aeruginosa with difficult-to-treat resistance (DTR-P. aeruginosa). *Clin Infect Dis* 72(7): e169-e183
11. Swain J, Dezanet C, Chalhoub H, Auquière M, Kempf J, Décout JL & Mingeot-Leclercq MP (2021). Interest of Homodialkyl Neamine Derivatives against Resistant P. aeruginosa, *E. coli*, and β-Lactamases-Producing Bacteria—Effect of Alkyl Chain Length on the Interaction with LPS. *Int J Mol Sci* 22(16): 8707.
12. Stryjewski ME & Sexton DJ (2003). Pseudomonas aeruginosa infections in specific types of patients and clinical settings. Severe infections caused by *Pseudomonas aeruginosa*. pp. 1-15.
13. Cowan ST (2004). Cowan and Steel's manual for the identification of medical bacteria. *Camb Uni Pre*.
14. CLSI C (2016). Performance standards for antimicrobial susceptibility testing. *CLSI* 35(3): 16-38.
15. Ahmad M, Hassan M, Khalid A, Tariq I, Asad MHHB, Samad A & Murtaza G (2016). Prevalence of extended spectrum β-lactamase and antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas* from patients of Khyber Pakhtunkhwa, Pakistan. *Biomed Res Int.*
16. Ullah F, Malik SA & Ahmed J (2009). Antimicrobial susceptibility and ESBL prevalence in *Pseudomonas aeruginosa* isolated from burn patients in the North West of Pakistan. *Burns* 35(7): 1020-1025.
17. Khan F, Khan A & Kazmi SU (2014). Prevalence and susceptibility pattern of multi drug resistant clinical isolates of *Pseudomonas aeruginosa* in Karachi. *Pak J Med Sci* 30(5): 951.
18. Mahmoud AB, Zahran WA, Hindawi GR, Labib AZ & Galal R (2013). Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in patients with nosocomial infections at a university hospital in Egypt, with special reference to typing methods. *J Virol Microbiol* 13: 165-59.
19. Sarwat T, Rashid M, Rastogi V & Chander Y (2015). A comparative study of Antibiogram of *Pseudomonas aeruginosa* in Hospital and community acquired infections. *Int J Curr Microbiol App Sci* 1: 286-291.
20. Shaikh S, Fatima J, Shakil S, Rizvi SMD & Kamal MA (2015). Prevalence of multidrug resistant and extended spectrum beta-lactamase producers *Pseudomonas aeruginosa* in a tertiary care hospital. *Saudi J Biol Sci* 22(1): 62-

64.
21. Ullah W, Qasim M, Rahman H, Bari F, Khan S, Rehman ZU & Muhammad N (2016). Multi drug resistant *Pseudomonas aeruginosa*: Pathogen burden and associated antibiogram in a tertiary care hospital of Pakistan. *Microb Pathogenesis* 97: 209-212.
22. Khan JA, Iqbal Z, Rahman SU, Farzana K & Khan A (2008). Prevalence and resistance pattern of *pseudomonas aeruginosa* against various antibiotics. *Pak J Pharm Sci* 21(3).
23. Tavajjohi Z & Moniri R (2011). Detection of ESBLs and MDR in *Pseudomonas aeruginosa* in a tertiary-care teaching hospital. *Iran J Clin Infect Dis* 6(1): 18-23.
24. Hosu MC, Vasaikar SD, Okuthe GE & Apalata T (2021). Detection of extended spectrum beta-lactamase genes in Pseudomonas aeruginosa isolated from patients in rural Eastern Cape Province, South Africa. *Sci Rep* 11(1): 1-8.
25. Samad A, Ahmed T, Rahim A, Khalil A & Ali I (2017). Antimicrobial susceptibility patterns of clinical isolates of *Pseudomonas aeruginosa* isolated from patients of respiratory tract infections in a Tertiary Care Hospital, Peshawar. *Pak J Med Sci* 33(3): 670.